Effect of Hyperlipidemia on Thymocyte Sensitivity to Apoptosis in CBA and C57Bl/6 Mice

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Effects of experimental hyperlipidemia on apoptosis and proliferation of thymocytes in response to mitogens were studied in CBA and C57Bl/6 mice. The concentrations of cholesterol in the serum and thymocyte membranes increased in both mouse strains. Spontaneous and dexamethasone-induced apoptosis *in vitro* and the proliferative response to phytohemagglutinin and concanavalin A were enhanced in thymocytes from C57Bl/6 mice and suppressed in cells from CBA mice. These data suggest opposite reactions of thymocyte to increased serum cholesterol concentration in these two strains, associated with stimulation and suppression of cell activity.

Key Words: *experimental hyperlipidemia; inbred mice; thymocytes; apoptosis*

Mice of different strains are characterized by different genetic predisposition to the development of lipid plaques in the aorta after atherogenic diets [10]. The genes responsible for the development of experimental atherosclerosis in mice were described (*Ath-1* [10] and *Ath-3* [12]). C57Bl/6 mice are considered to be the most sensitive to aortic damage, but no correlation between blood cholesterol and sensitivity to the formation of aortic lesions were found. CBA mice also react to atherogenic diet by elevation of serum cholesterol [11]. On the other hand, the immune response of CBA and C57Bl/6 mice to foreign antigens is different [4]. We previously showed that atherogenic diet suppressed immune response to sheep erythrocytes in CBA mice and stimulated it in C57Bl/6 mice [6].

Atherosclerotic process is associated with accumulation of atherogenic lipids in the blood and cholesterol in lymphocyte membranes [3]. Cholesterol acts as a molecular modifier of membranes altering their fluidity and increasing membrane rigidity [15]. The state of plasma membrane is essential for apoptosis. We investigated the effect of experimental hyperlipidemia on apoptosis and proliferation of thymocytes in response to mitogens *in vitro*.

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MATERIALS AND METHODS

Experiments were carried out on CBA and C57Bl/6 mice (18-20 g) from Rappolovo Breeding Center (Russian Academy of Medical Sciences). Experimental mice were fed atherogenic diet (67% standard granulated fodder, 3% cholesterol, 28.8% sunflower oil, and 0.2% cholic acid) for 2 months and controls were given a standard ration. The content of total and α-cholesterol and triglycerides were measured on an AA-2 analyzer (Technicon). Lipid composition of thymocyte membranes was studied by gas chromatography [3]. The percentage of apoptotic cells was counted under a fluorescent microscope after acridine orange and ethidium bromide staining as described previously [7]. Thymocyte apoptosis without preincubation, spontaneous (without inducer), and induced with 5 μM dexamethasone after 3-h incubation at 37°C in medium 199 with 10% fetal calf serum were evaluated. DNA fragmentation was evaluated by diphenylamine (DPA) test [7]. Thymocytes were preincubated for 24 h under the same conditions without inducer or with 5 μM dexamethasone and 1 μg/ml phorbol ester. For evaluation of

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the proliferative activity, the thymocytes were cultured for 72 h with 10 μg/ml phytohemagglutinin (PHA, Serva) or 5 μg/ml concanavalin A (ConA, Pharmacia) [1]. The data were processed statistically using Student's *t* test.

RESULTS

Hyperlipoproteinemia manifesting by accumulation of total and α-cholesterol developed after 2-month atherogenic diet in mice of both strains, but the total cholesterol content was higher in CBA mice (Table 1). The content of cholesterol in thymocyte membranes also increased in both animal strains, but the content of free cholesterol was much higher in CBA mice: it increased two-fold in the control and 1.7 times in the experimental group.

Fluorescent test demonstrated stimulation of apoptosis in CBA and its suppression in C57Bl/6 mice. These data were confirmed by studies of DNA fragmentation in DPA test in CBA mice (Table 1). The sensitivity to apoptosis correlated with changes in thymocyte proliferative activity: the response to mitogen was suppressed in CBA and enhanced in C57Bl/6 mice (Table 1). The weight of the thymus did not change and was 48.5±5.1 and 45.3±4.2 mg in CBA and 37.8±3.9 and 32.2±2.3 mg in C57Bl/6 mice in the control and experimental groups, respectively.

Apoptosis is an important mechanisms of T-lymphocyte maturation and differentiation. Both stimulation and suppression of thymocyte apoptosis lead to negative consequences and impair normal formation of thymocytes. Our data are in line with the results of studies with thymocyte incubation *in vitro*. A dose-dependent effect was observed with increasing the concentration of exogenous cholesterol: high percentage of apoptotic cells at the beginning and then blockade of apoptosis [5,9]. A dose-dependent effect was also observed in studies of lipoprotein effect on proliferative activity of mitogen-stimulated mouse splenocytes [14].

The state of cell membrane and intensity of LPO are essential for initiation of apoptosis [9]. Additional incorporation of cholesterol into the membrane cancels the point of phase transition and increases the rigidity of fatty acid chains in phospholipids [15], which can impede oxygen diffusion and suppress LPO [5]. Changes in membrane fluidity and metabolism can cause imbalance of second messengers, in particular lead to activation of protein kinase C [9]. There are data that LDL activate protein kinase C in cell culture

TABLE 1. Effect of Atherogenic Diet on Lipid Content in Serum and Thymocyte Membranes and on Thymocyte Apoptosis and Proliferation in CBA and C57Bl/6 Mice (*M*±*m*, *n*=8)

Parameter	CBA		C57Bl/6	
	control	experiment	control	experiment
Serum lipids, mmol/liter:				
total cholesterol	2.4±0.1	6.0±0.3*	2.3±0.1	3.8±0.2*
α-cholesterol	1.6±0.1	3.0±0.2*	1.2±0.1	1.5±0.1***
triglycerides	1.6±0.1	1.7±0.1	0.8±0.1	1.1±0.1***
Thymocyte lipids:				
total cholesterol, μg/mg protein	17.6±0.1	26.0±0.2*	10.3±0.1	18.4±0.1*
free cholesterol, μg/mg protein	17.2±0.1	22.7±0.4*	8.6±0.2	13.1±0.4**
cholesterol esters, %	3.1±0.2	12.7±0.7*	11.5±1.1	29.2±2.4*
Thymocyte apoptosis, %:				
without incubation	6.5±0.6	3.7±0.6**	4.2±0.4	16.1±1.5*
after 3-h incubation	20.0±1.8	11.5±1.9*	15.7±1.6	21.8±2.4***
after 3-h incubation with dexamethasone	28.2±1.8	21.6±1.3**	23.4±1.9	35.5±2.0*
DNA fragmentation, %:				
after 24-h incubation	51.0±2.3	42.6±2.7***	31.5±2.7	29.2±2.3
after 24-h incubation with dexamethasone and phorbol ester	72.0±2.7	62.3±1.8***	60.4±1.6	57.4±2.5
Thymocyte proliferation, cpm:				
PHA-stimulated	40 604+4562	24 317±3451**	4271±573	5941±642***
ConA-stimulated	170 059+19 641	82 996±9681**	9621±1016	65 484±6487**

Note. *$p<0.001$, **$p<0.01$, **$p<0.05$ *vs.* the control.

[13]. Polyunsaturated fatty acids of sunflower oil can also modify membrane fluidity and directly activate protein kinase C [8].

These data suggest opposite types of thymocyte reaction to increased cholesterol content in different mouse strains. Presumably the cell less saturated with cholesterol better responds to stimuli, while saturation of membrane inhibits cell reaction. Enhanced thymocyte apoptosis can lead to preservation of the undesirable clones and to the development of autoimmune reactions, in particular atherosclerosis, which can be an immunopathological state [2].

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REFERENCES

1. *Methods of Immunology*, Ed. G. Friemel [in Russian], Moscow (1990).
2. A. N. Klimov and N. G. Nikul'cheva, *Lipids, Lipoproteins, and Atherosclerosis* [in Russian], St. Petersburg (1995).
3. I. G. Kovaleva and V. M. Dil'man, *Fiziologiya Cheloveka*, **7**, No. 1, 120-124 (1981).
4. R. V. Petrov, R. M. Khaitov, V. M. Man'ko, *et al., Immune Response Control and Regulation* [in Russian], Leningrad (1981).
5. V. S. Posokhov, O. A. Rozenberg, and K. P. Khanson, *Byull. Eksp. Biol. Med.*, **113**, No. 2, 136-137 (1992).
6. V. P. Puzyreva, R. P. Ogurtsov, A. V. Polevshchikov, *et al., Lab. Zhivotnye*, **2**, 90-98 (1995).
7. *Current Protocols in Immunology*, Eds. J. E. Coligan *et al.*, New York (1992), vol. 1, pp. 1-16.
8. H. W. De Jonge, D. H. Dekkers, and J. M. Lamers, *Mol. Cell. Biochem.*, **157**, No. 1-2, 199-200 (1996).
9. S. Hartel, F. Ojeda, and H. Diehl, *Int. J. Radiat. Biol.*, **74**, No. 5, 607-615 (1998).
10. B. Paigen, D. Mitchell, K. Reue, *et al., Proc. Natl. Acad. Sci. USA*, **84**, 3763-3767 (1987).
11. A. Roberts and J. S. Thompson, *Progr. Biochem. Pharm.*, **13**, 298-305 (1977).
12. J. L. Stewart-Philips, J. Lough, and E. Skamene, *Clin. Invest. Med.*, **12**, No. 2, 121-126 (1989).
13. R. K. Studer, P. A. Craven, and F. R. DeRubertis, *J. Lab. Clin. Med.*, **125**, No. 1, 86-95 (1995).
14. E. R. Waelti, R. Glueck, and M. W. Hess, *Eur. J. Immunol.*, **11**, No. 10, 846-848 (1981).
15. P. L. Yeagle, *Biochim. Biophys. Acta*, **822**, 267-287 (1985).